COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Stuart A. Aaronson of Mount Sinai Medical Center, New York, New York, United States of America, declare as follows:

1. I am currently the Director of the Dorald H. Ruttenberg Cancer Center for the Mount Sinai Medical Center in New York, New York. I have held this position since 1993. From 1977 to 1993, I was the Chief of the Laboratory of Cellular and Molecular Biology at the National Cancer Institute, Bethesda, Maryland. From 1970 to 1977, I was the Head of the Molecular Biology Section, from 1969 to1970, a Senior Staff Fellow and from 1967 to 1969, a Staff Associate at the Viral Carcinogenesis Branch at the National Cancer Institute. I was awarded my M.D. in 1966 from the University of California Medical School, San Francisco.

2. Since the 1970s, my research has focused on growth factors and their role in tumorigenesis and cancer as evidenced by my curriculum vitae, which lists the publications that I have authored or co-authored. My research in the area of the molecular biology of growth factors and their receptors, including keratinocyte growth factor, fibroblast growth factor and vascular endothelial growth factor, has encompassed mammalian models of tumorigenesis,

including human tumor model systems. Now shown to me and marked "Annexure 1" is a copy of my curriculum vitae.

3. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to review Australian Patent Application Au-B-696764 (73941/94) in the name of HGS, entitled "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), which claims priority and has a virtually identical specification to U.S. application no. 08/207,550, filed March 8, 1994. I have been asked to review and comment on the experimental evidence provided in Dr. Alitalo's Statutory Declaration. I have also been asked to review and comment on the experimental evidence provided in a draft of Dr. Susan Power's Statutory Declaration, which the Patent Attorneys representing HGS have stated she will be serving in these proceedings. I have also been asked to provide my comments and opinions as to what the patent specification would provide to one of ordinary skill in the art of the molecular biology of growth factors as of the earliest filing date of the HGS patent specification, March 1994. For purposes of this analysis, I considered not only what I knew and appreciated at the relevant time, but what was expected to be known by graduate students and postdoctoral fellows who were in my laboratory at the relevant time.

Specific Comments Concerning The Patent Specification

4. I have reviewed and analyzed the polynucleotide, and amino acid sequence, identified by HGS to encode the human VEGF-2 protein, as set forth in Figure 1 of the HGS patent specification. The HGS patent specification describes, but is not limited to, the characterization of the VEGF-2 sequence and encoded protein. The HGS patent specification describes the human VEGF-2 protein as structurally related to the PDGF/VEGF family, a known family of secreted growth factors. The HGS patent specification further discloses that the VEGF-2 polynucleotide is predicted to contain an open reading frame of

approximately 1050 residues, which encodes VEGF-2. (See, the HGS patent specification at page 5, lines 25-27). The specification reports that at the amino acid level, VEGF-2 exhibits the highest homology to vascular endothelial growth factor (30% identity), followed by PDGF alpha (23%) and PDGF beta (22%). (See, the HGS patent specification at page 5, lines 28-31). The HGS patent specification further characterizes the VEGF-2 protein as containing eight cysteines which are conserved among all known members of the PDGF/VEGF family, and in addition, also contains the fourteen amino acid signature motif, PXCVXXXRCXGCCN, found in all members of the PDGF/VEGF family. (See, the HGS patent specification at page 5, lines 31-33). The HGS patent specification speculates that the first 24 residues of the 350 amino acid sequence may encode a signal sequence. (See, the HGS patent specification at page 4, lines 29-31, and page 5, lines 26-27).

5. Based on the characterization of the VEGF-2 protein set forth in the HGS patent specification, one would recognize that the protein was a member of the PDGF/VEGF family of growth factors. The PDGF/VEGF family of growth factors, like other growth factors, must be secreted in order to exert their growth promoting or mitogenic effects. Since all previously identified members of the PDGF/VEGF family were known to be secreted, one would expect the newly identified VEGF-2 to also be secreted.

6. By March 1994 it was well known to me and, I believe to my colleagues in the angiogenic field that the PDGF/VEGF family of growth factors were expressed initially as precursor proteins which underwent proteolytic processing resulting in a mature, secreted form of the protein. Thus, I would have predicted that VEGF-2 would be expressed in a similar way. The 350 amino acid sequence set forth in Figure 1 of the HGS patent specification contains the conserved, signature motifs for an active form of a protein belonging to the PDGF/VEGF family. Thus in March 1994, I would have predicted the protein encoded by the sequence disclosed in Figure 1 of the

HGS patent specification, containing those signature motifs characteristic of the PDGF/VEGF family to be biologically active.

Specific Comments on Experimental Evidence Supplied In Dr. Alitalo's Declaration

7. Dr. Alitalo's Declaration describes experiments designed to determine whether the 350 amino acid sequence disclosed in the HGS patent specification is secreted as a mature form of the VEGF-2 protein. These experiments utilize constructs encoding amino acids 70 to 419 of the full length VEGF-2 (i.e., amino acids 1 to 350 described in the HGS patent specification) modified to contain a hemagglutinin peptide tag (HA) fused to its carboxy terminus or a vector encoding the complete 419 amino acid sequence of VEGF-C.

8. These vectors were transiently transfected into a mammalian cell line, 293T cells. The cell lysates and culture medium were assayed for the presence of newly synthesized VEGF-2/VEGF-C proteins. VEGF-2/VEGF-C were partially purified from the cell medium and cell lysates using an immunoprecipitation procedure using antibodies. The antibody used to detect VEGF-2 (70-419) was a monoclonal antibody that recognizes the hemagglutinin peptide tag. By contrast, the antibody used to immunoprecipitate VEGF-C (1 419) was a polyclonal antibody which recognizes residues 31 to 51 of the 350 amino acid VEGF-2 polypeptide. At the outset, I note that serious flaws are introduced into Dr. Alitalo's experimental design given that two different antibodies were used in the study.

9. First, Dr. Alitalo has reported the inability to isolate VEGF-C using an antibody which recognizes the C-terminal residues 372-394 or a tag attached to the C-terminus of VEGF-2 (Joukov et al., 1997, EMBO J 16: 3898, at 3900, "Joukov"). As Dr. Alitalo has reported, the amino and carboxy terminal propeptides of the precursor form of VEGF-C/VEGF-2 undergo extensive proteolytic processing, resulting in the mature form of the protein (see, Joukov

at pages 3906-7). Dr. Alitalo has also reported that the carboxy terminal propeptide "is cleaved additionally at its C-terminus," accounting for his inability to isolate VEGF-C either by using an antiserum against the C-terminal amino acid residues 372-394 or by using a tag at the C-terminus (see Joukov at page 3900, second column). If VEGF-2 is efficiently proteolytically processed to the mature form, the tag is cleaved from the carboxy terminus. The tag no longer linked to the mature form of VEGF-2 does not allow for the isolation and detection of the mature protein. Thus, the mature secreted form would not be detected using an antibody which recognizes a tag attached to the carboxy terminus, as reflected by Dr. Alitalo's own publications.

10. The HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA-tag at its carboxy terminus using a monoclonal antibody to HA (See, HGS Australian Patent No. 714484 and Hu J.S. et al. FASEB J. 11 (6): 498-504). However, the HGS studies were conducted in COS cells, whereas Dr. Alitalo's experiments were conducted in 293T cells (see Dr. Alitalo's Declaration at ¶ 6.2). The significance of the different cell types used is provided by Dr. Alitalo's own publications (Joukov). This publication compares the proteolytic processing of VEGF-2 expressed by a number of different cell lines, including COS cells, PC-3 cells, HT 1080 cells, and 293 EBNA cells. The results of this comparison, as reported by Dr. Alitalo was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types." (Joukov, at page 3901, second column). Thus, as the VEGF-C precursor is processed less efficiently in COS cells, the cleavage of the HA tag from the carboxy terminus should also be less efficient, which may account for HGS's ability to successfully isolate the protein from COS cells using a carboxy HA tag.

11. Second, the use of two antibodies introduces another serious flaw into the experimental design, which in the absence of the appropriate controls prevents

drawing meaningful conclusions from the data obtained. Since the antibodies recognize completely different determinants -- one within VEGF C and the other an HA-peptide tag -- each will have different affinities for the proteins they bind. Furthermore, one antibody is polyclonal and the other is monoclonal. Consequently, it is not possible to make any quantitative comparison between the results obtained for VEGF-C and VEGF-2, since the differing efficiency with which the antibodies bind their targets does not allow direct comparison of the level of proteins present in the assayed samples.

12. The aim of Dr. Alitalo's study is to determine whether VEGF-2 (HGS) is processed correctly and secreted by a mammalian cell line. However, Dr. Alitalo's experimental design allows for the detection of both the precursor and mature processed forms of VEGF-C with an anti-VEGF-C antibody; whereas the antibody used to detect VEGF-2 recognizes an HA tag fused to the C-terminus of the unprocessed form of VEGF-2 (see, Exhibit 3 of Dr. Alitalo's Declaration). As discussed in ¶ 9 above, efficient processing of the precursor protein results in extensive processing of both the amino and carboxy terminal ends thus, preventing detection of the mature protein with a carboxy terminal tag. However, the precursor form of VEGF-2 which still retains the HA tag is readily detectable (see Exhibit 3 of Dr. Alitalo's Declaration). Thus, Dr. Alitalo's experimental design allows for the detection of both unprocessed precursor form and mature VEGF-C, whereas only the unprocessed precursor form of VEGF-2 can be detected.

13. Therefore, it is not possible to draw any meaningful conclusions about the relative efficiency of secretion of VEGF-C and VEGF-2 (70 to 419) from these data – the same antibody should have been used in both cases. Furthermore, no controls have been carried out to determine the transfection efficiency of the plasmids used -- thus preventing any valid quantitative comparisons being drawn from the data obtained.

14. Dr. Alitalo states that the expression level of VEGF-C is much higher than that of VEGF-2 (HGS), and this may be due to inefficient translation and/or that the intracellular turnover rate of VEGF2 (HGS) is much faster than that of VEGF-C (Dr. Alitalo's Declaration at ¶ 8.3). Neither of these conclusions are supported by the data set forth in Dr. Alitalo's Declaration. As discussed in ¶¶ 9 to 12 above, it is not possible to draw any conclusions from these data based on flawed quantitative comparisons. The conclusions set forth in Dr. Alitalo's Declaration are mere speculation that are unsupported by the results obtained.

Specific Comments on Experimental Evidence Supplied in Dr. Power's Declaration

15. Thus, the question of whether the 350 amino acid sequence as set forth in the HGS patent specification does indeed contain sufficient information to result in the mature processed form of VEGF-2 when secreted from a cell has, in my opinion, been addressed and affirmatively confirmed in the experiments reported in Dr. Susan Power's Statutory Declaration.

16. By March 1994, had I found that the VEGF-2 350 amino acid sequence set forth in the HGS patent specification was not secreted, in order to ensure secretion of VEGF-2, I would have engineered a heterologous signal sequence upstream and in frame with the 350 amino acid sequence, and it would have involved routine practice to do so. Indeed, this approach is specifically taught in the HGS patent specification (at page 14, lines 6-23).

17. The expectation that I and, I believe others of ordinary skill in the field would have had, that engineering a signal sequence upstream of the sequence set forth in Figure 1 of the patent specification would result in the expression and secretion of a biologically active protein as set forth in the HGS patent specification, has indeed been confirmed by the experimental evidence provided in Dr. Power's Declaration. The experiment set forth in Dr. Power's

Declaration describes the use of two constructs, the 350 amino acid sequence of VEGF-2 (as set forth in Figure 1 of the patent specification) fused in frame with a heterologous signal sequence and the 419 amino acid sequence of VEGF-2. These two constructs were used to transform a mammalian cell line. The cells were cultured under conditions to allow the cells to express the gene products encoded by the vectors. At various time points the cell lysates and culture medium were collected and each was assayed for the presence of VEGF-2. The presence of VEGF-2 was determined by a Western blot analysis using according to Dr. Power's Declaration, a polyclonal antibody to VEGF-2 that recognizes both the unprocessed precursor form as well as the processed, secreted form of VEGF-2 (See ¶ 13 of Dr. Power's Declaration).

18. Further, in my opinion, the flaws in Dr. Alitalo's experimental design which prevented any meaningful conclusions being drawn from the data presented, have been addressed in the experiments reported in Dr. Power's Declaration. First, the same antibody was used to detect the 350 amino acid sequence and the 419 amino acid sequence. Furthermore, the anti-VEGF-2 antibody used in the study is capable of detecting both the precursor form and the mature, processed form of VEGF-2. Second, the experimental protocol included comparing transfection efficiencies, and also included positive and negative controls for the expression of VEGF-2 (i.e., the expression vector encoding full-length VEGF-2 and the expression vector in the absence of any VEGF 2 sequence). In addition, the experimental protocol allowed for detection of VEGF-2 protein expression and secretion over a 72 hour time period.

19. The results of the experiments described in Dr. Power's Declaration clearly demonstrate that the 350 amino acid sequence of VEGF-2 fused in frame with a heterologous signal sequence results in the secretion of VEGF-2 from the cell (see Figure 1 of Dr. Power's Declaration). The secreted product resulting from both the 350 signal sequence construct and the 419 amino acid sequence construct, resolves as a broad band of approximately 30 kDa, and at the later

time points, e.g., 72 hours, one can also detect, in addition to this band, a minor band of approximately 21 kDa secreted by both constructs (see Figure 1 of Dr. Power's Declaration, in particular at Gel 3, lanes 22 and 24). The observation of a broad band at approximately 30 kDa and a minor band at approximately 21 kDa is consistent with Dr. Alitalo's observation that the majority of secreted VEGF-C is detected as a broad doublet band of approximately 29-31 kDa and another minor band of about 21 kDa (see, Alitalo Declaration at ¶ 7.2). Dr. Alitalo's publications also confirm that a doublet at approximately 30 kDa and another band at 21 kDa reflects a correctly processed form of VEGF-2 (see Joukov at page 3898). Thus, the results present in Dr. Power's Declaration confirm that engineering a heterologous signal sequence in frame with the coding sequence of the 350 amino acid sequence set forth in Figure 1 of the patent specification, not only results in the secretion of the protein into the culture medium, but also results in the secretion of a correctly processed mature form of VEGF-2.

20. The results of the experiments reported in Dr. Power's Declaration also demonstrate that the expression of the 350 amino acid sequence of VEGF-2 with a signal sequence contains sufficient information to allow for the correct processing of the protein to a mature biologically active protein. The expression of the 350 amino acid form of VEGF-2 results in the secretion of a proteolytically processed protein which is the same size as the secreted processed form resulting from the expression of the 419 amino acid construct. The secreted proteins which result from the expression of the 419 amino acid form of VEGF-2 and the 350 amino acid form of VEGF-2 with a signal sequence are indistinguishable in size. Both are secreted as a protein which resolves as a band at approximately 30 kDa, with another minor band detectable at approximately 21 kDa (see Figure 1 of Dr. Power's Declaration, at Gel 3, lanes 22 and 24). This is in agreement with Dr. Alitalo's observations of the mature biologically active form of VEGF-C as set forth in his declaration and publication (see ¶ 19 above). Thus, consistent with the

teaching of the HGS patent specification, the 350 amino acid sequence with a signal sequence, as set forth in the patent specification, contains sufficient information to result in the correct proteolytic processing of the VEGF-2 protein.

21. The expectation that the sequence set forth in Figure 1 of the patent specification does indeed contain the conserved motifs which would confer biological activity to the secreted VEGF-2 protein has also been confirmed by the experimental evidence provided by Dr. Power's Declaration. The results presented in Dr. Power's Declaration clearly demonstrate the 350 amino acid sequence of VEGF-2 fused in frame with a signal sequence results in the secretion of a proteolytically processed form which is resolved as a band at approximately 30 kDa and another minor band at approximately 21 kDa, the same species observed with expression of the 419 amino acid form of VEGF-2 (see Dr. Power's Declaration, Figure 1 at Gel 3, lines 22 and 24). The 30 kDa and the 21 kDa species have been consistently identified in the art as the correct processed form of VEGF-2 with biological activity. Dr. Alitalo identifies this same species as the mature biologically active form in his own declaration (see, Dr. Alitalo's Declaration at ¶ 7.2). Dr. Alitalo also identifies this same species as the mature biologically active form of VEGF-2 in his publications (see, Joukov at page 3900). Thus, the secreted VEGF-2 protein resulting from the expression of the 350 amino acid sequence set forth in the patent specification, would also be expected to have biological activity.

22. In sum, the experimental evidence provided in Dr. Power's Declaration demonstrates that the 350 amino acid sequence disclosed in the HGS patent specification fused in frame with a signal sequence results in a secreted form of VEGF-2; and that the 350 amino acid sequence contains sufficient information to be correctly processed by the cell. Furthermore, the expression of the 350 amino acid sequence with a signal sequence results in the secretion of a mature form of VEGF-2, which is indistinguishable from that observed

with expression of the 419 amino acid form of VEGF-2 as shown by both Drs. Power and Alitalo.

Conclusion

23. In my opinion, I or one skilled in the art would identify the VEGF-2 protein as a novel member of the PDGF/VEGF family of growth factors, and as such, would recognize that VEGF-2 is also a growth factor, based on the HGS patent specification in combination with the state of the art as of March 1994. I or one skilled in the art following the teaching of the HGS patent specification coupled with the state of the art, would predict that the 350 amino acid sequence with a signal sequence would result in the expression and secretion of a protein which retains VEGF-2 biological activity.

24. The experimental evidence provided in Dr. Power's Declaration confirms the teachings of the HGS patent specification, demonstrating that the 350 amino acid sequence set forth in the patent specification fused in frame to a signal sequence results in a secreted form of VEGF-2, which is correctly processed by the cell. Furthermore, these results confirm that the 350 amino acid sequence with a signal sequence contains sufficient information to be correctly processed by the cell resulting in a secreted, biologically active VEGF-2 protein.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Stuart A. Aaronson, Stuart A Aaronson,
at New York, New York, on this 14th day of December 2000;
before me: Maryann White.
Notary Public

MARYANN WHITE
NOTARY PUBLIC, State of New York
No. 4883761
Qualified in Nassau County
Certification Filed in New York County
Commission Expires January 26, 2001

CURRICULUM VITAE

Name: Stuart A. Aaronson

Date and Place of Birth: February 28, 1942, Mt. Clemens, Michigan

Citizenship: U.S.A.

Marital Status: Married, three children

Education and Training:

1959-1962	B.S. (Chemistry; summa cum laude), University of California, Berkeley
1962-1966	M.D., University of California Medical School, San Francisco
1965-1966	Fellowship, Dept. of Biochemistry, University of Cambridge, Cambridge, United Kingdom
1966-1967	Intern, Medicine, Moffitt Hospital, San Francisco

Brief Chronology of Employment:

1967-1969	Staff Associate, Viral Carcinogenesis Branch, National Cancer Institute, Bethesda, MD
1969-1970	Senior Staff Fellow, Viral Carcinogenesis Branch
1970-1977	Head, Molecular Biology Section, Viral Carcinogenesis Branch
1977-1993	Chief, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland
1993-	Director, Ruttenberg Cancer Center, Mount Sinai Medical Center, New York, NY & Jane B. and Jack R. Aron, Professor of Neoplastic Diseases

Medical Licenses

New York
Virginia

Honors and Awards:

1962	Phi Beta Kappa
1966	Alpha Omega Alpha
1982	Rhoads Memorial Award
1982	PHS Meritorious Service Medal
1989	Paul Ehrlich Award
1989	PHS Distinguished Service Medal
1990	Milken Award
1991	Chirone Prize
1991	Harvey Lecture

1991 Wadsworth Memorial Foundation Award

Societies:

American Society for Microbiology
American Association for the Advancement of Science
Society for Experimental Biology and Medicine
American Association for Cancer Research, Inc.
American Society for Virology, Inc.

Memberships and Affiliations:

1975-1978 Member, Viral Cancer Program Coordinating Committee
1975-1976 Ad Hoc Member, Experimental Virology Study Section, NIH
1975-1978 Member, Viral Oncology Scientific Advisory Committee for FCRC
1976-1980 Member, Experimental Virology Study Section, NIH
1977- Member, Editorial Board, International Journal of Cancer
1977-1986 Associate Editor, Journal of the National Cancer Institute
1980-1985 Editorial Advisory Board, Biochimica et Biophysica Acta (BBA Reviews on Cancer)
1981- Associate Editor, Cancer Research
1983- Executive Committee, Duke Comprehensive Center, Duke University Medical Center
1984 Mott Selection Committee, General Motors Cancer Research Foundation
1984- Advisory Committee, Maimonides Conferences on Cancer Research
1984-1990 Editorial Board, Virus Research
1984-1987 Scientific Advisory Committee, American Cancer Society
1985-1987 External Scientific Review Committee, Comprehensive Center, The University of Alabama in Birmingham
1985- Editorial Advisory Board, Cancer and Metastasis Reviews
1985- Editorial Board, Cancer Reviews
1985-1989 Councillor, Society for Experimental Biology and Medicine
1985-1990 Extramural Advisory Board, Cancer Center, The University of Arizona
1986 Program Chairman, American Association of Cancer Research
1986 Co-organizer, Princess Takamatsu Symposium
1986- Guest Editor, Japanese Journal of Cancer Research (Gann)
1986- Editorial Board, Environmental and Occupational Health Sciences
1986-1987 Member, Advisory Committee, American Type Culture Collection
1987-1989 Editorial Advisory Board, Molecular Endocrinology
1987- Editorial Board, Oncogene
1988-1989 Advisory Editorial Board, ISI Atlas of Science: Biochemistry
1988- Member, Blood Services Scientific Council, American Red Cross
1989-1991 Editorial Board, Cancer Communications

1989-1992 Editorial Board, The New Biologist
1989 Visiting Professor, University of Texas, San Antonio
1990- Advisory Board, BBA Reviews on Cancer, Biochimica et Biophysica Acta
1990- General Motors Visiting Professor, University of Wisconsin-Madison Medical School
1990- Visiting Professor, Jonsson Comprehensive Cancer Center, University of California, Los Angeles
1992- Editorial Board, Intl. Journal of Oncology
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1993-1995 Editorial Advisory Board, Molecular Aspects of Medicine
1994- International Advisory Board, Tumori
1995-1996 Vice President, Harvey Society
1995- External Scientific Advisory Committee, UCLA Oral Cancer Center
1996-1997 President, Harvey Society
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1998 Member, Public Relations and Communications Committee, AACR
1998 Member, The National Neurofibromatosis Foundation Research Advisory Board

Research Interests:

Molecular genetics of cancer; retrovirology; cellular growth regulation by growth factors and their receptors.

Patents:

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